Remarks

Applicants wish to thank the Examiner for the telephonic conversation with the undersigned on August 13, 2002 in which the basis of the Examiner's rejection was discussed. Applicants and the Examiner have a different interpretation of claims 1 and 48 and the remarks herein address those differences. Applicants have removed the phrase "for transfecting higher eucaryotic cells with nucleic acid molecules *in vitro* and *in vivo*" from the preamble of claims 1 and 48 to make it clear that this phrase was merely an intended use recitation which was not intended to be construed as conferring an additional limitation in the composition claims.

Upon entry of the foregoing amendment, claims 1-33, 37-41, 45, 46 and 48 are pending in the application, with claims 1 and 48 being the independent claims. Claims 34-36, 42-44 and 47 have been cancelled solely to expedite prosecution of the composition claims. Claims 15 and 17 were amended to more clearly define the cationic precursor molecules claimed therein. Support for these changes can be found on pages 42 and 57 of the Written Description respectively. The Examiner's attention is directed to claims 8-11 which have been amended to include guanidyl and ornithylamino so that they encompass the claimed subject matter of dependent claims 12 and 14-17. One of ordinary skill in the art would recognize that the claims from which claims 12 and 14-17 depend must include these particular residues. The alkylene group of R_2 of claim 8 has also been amended to include up to four amino radicals $-NR_4R_5$ such that claim 8 encompasses the claimed subject matter of dependent claims 9-11. One of ordinary skill

in the art would recognize that claim 8 must include these particular radicals as they are recited in dependent claims 9-11. Support for the changes made to claim 12 and 16 can be found in the Written Description, for example, at page 57. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Applicants' Invention

At least one critical feature which distinguishes the claims from the prior art also enables the full scope of those claims. In particular, the prior art does not anticipate, teach or suggest transfection particles comprising organic cationic precursor molecules *intermolecularly bonded* to other such molecules. Whereas such molecules in the claimed particles are covalently bonded to each other, they are only ionically associated with the nucleic acid molecules. The claimed precursor molecules' capability of forming these intermolecular bonds is critical in facilitating the formation of Applicants' claimed transfection particles: "The general concept of the invention *requires* one or more at least bifunctional precursor molecules that are on the one hand cationic in order to complex nucleic acid molecules essentially without aggregating or crosslinking it, and on the other hand capable of covalently linking to identical or different cationic precursor molecules of the particle structure for stabilizing that particle." *See* Specification, page 3, last paragraph, emphasis added. The intermolecular covalent bonds facilitate the

formation of stable transfection particles. *See* Specification, page 3, lines 3-5 and figure 1. Applicants' inventive precursor molecules improve and enable the ability to form stable nucleic acid transfection particles.

Rejections under 35 U.S.C. § 112

The Examiner has rejected claims 1-48 as not being enabled by the specification. PTO File Wrapper Paper No. 14, page 3, paragraph 4. This rejection is based on two allegations: i) the state of the art demonstrates a high level of unpredictability for making liposomes that comprise detergents and nucleic acid molecules; and, ii) the claims are not enabled because there is no expectation of success that they can be used *in vivo*. S*ee Id.* at page 3, para. 4, lines 4-8; page 6, line 18 to page 7, line 9; and page 9, lines 5-12. Applicants respectfully traverse the § 112 rejection.

Applicants' Written Description Enables the Claims

Applicants have provided sufficient guidance to enable the skilled artisan to make the full scope of claims 1 and 48. These claims are directed to transfection particles comprising one or more cationic molecules complexed to nucleic acid, wherein the cationic molecules are obtained by linking precursor molecules to each other via covalent bonds. A method of making the claimed genus of transfection particles is provided in the Written Description from the fifth full paragraph on page 26 through the second full paragraph on page 28. Guidance as to the cationic precursor molecules used to make the transfection particles is given, for example, from pages 5-6 and 8-18 in the written

description; and guidance as to the nucleotide molecules used to make the transfection particles is given, for example, in pages 25-26.

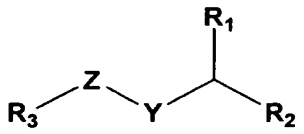
Moreover, Applicants are not required to exhaustively provide experimental guidance for every potential transfection particle. Rather, the USPTO has promulgated guidelines explicitly stating that

> [f]or a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation.

M.P.E.P. (Eighth) §2164.02 (Working Examples and a Claimed Genus) (2001).

Here, the claimed genus of transfection particles all share properties with the working examples described in pages 37-79 of the written description (e.g., condensed nucleic acid complexed to precursor molecules having a function for ionic interaction with the nucleic acid and a function to form covalent bonds with other precursor molecules). These examples also enable the formation of the claimed transfection particles having intermolecularly bonded precursor molecules.

Claim 8 is narrower in scope than claims 1 and 48 and is also certainly enabled by Applicants' Written Description. Claim 8 requires that the organic cationic precursor molecule is represented by general formula I



(I)

wherein R_1 denotes (C_1-C_{10}-alkylene)-SH; R_2 denotes -NR_4R_5, -$NHR_4R_5^+$, -$N(R_4)_2R_5^+$, -$C(=NR_4)NR_5R_6$, guanidyl, ornithylamino, or -C(=X)-C_1-C_{10}-alkylene, wherein the alkylene radical may represent a straight chained or branched hydrocarbon and may be substituted by up to four amino radicals -NR_4R_5 or a thiomonosaccharide; R_3 denotes particular lipophilic groups; R_4, R_5 and R_6 denote independently from each other hydrogen or C_1-C_4-alkyl; X denotes O or S; Y denotes C=O or C=S and Z denotes O, S or -NR_4-. Hence, claim 8 encompasses a genus of transfection particles having a precursor molecule defined by I. The claimed genus of precursor molecules and the representative examples described in the Written Description share an alkylene-SH group at R_1, a particular group to complex nucleic acid at R_2, and a particular lipophilic residue at R_3. The skilled artisan would expect that this claimed genus of precursor molecules could be used to make transfection particles without undue experimentation. Accordingly, Applicants' Written Description enables claim 8.

Applicants have also provided sufficient guidance to enable the skilled artisan to use the full scope of the claimed invention. The legal standard for enablement as it relates to composition claims can be found in the M.P.E.P.:

> . . . when a compound or composition claim is not limited by a recited use, any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use. If multiple uses for claimed compounds or compositions are disclosed in the application, then an enablement rejection must include an explanation, sufficiently supported by the evidence, why the specification fails to enable each disclosed use. In other words, if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention.

M.P.E.P. (Eighth) §2164.01(c) How to Use the Claimed Invention (2001). Thus, Applicants need only demonstrate one use in order to enable the composition or

composition comprising kit claims 1-33, 37-41, 45, 46 and 48. Such use can be found in the Written Description or can be shown to be a use well known to those of ordinary skill in the art.

Here, the first paragraph of Applicants' Written Description provides at least one example of such use: "new transfection particles that are useful for transfecting higher eucaryotic cells *in vitro*. . . ." This use is demonstrated in the working examples provided in the Written Description from pages 37-79. Moreover, the Examiner has acknowledged this use: ". . . it is agreed that the method of transfecting cells in cell culture is not a serious burden on one of skill in the art. Although the exact concentration of liposomes must be determined so that the transfection is not toxic, such assays are easily performed by use of concentration gradients in the laboratory." *See* PTO File Wrapper Paper No. 14, page 17, lines 9-12. (See also *Id.* at page 19, lines 18-19: ". . . it is not disputed that liposomes are well-known for in vitro transfection.") Hence, the composition and composition comprising kit claims are enabled with respect to using the transfection particles.

The Examiner Has Not Made a Prima Facie Showing of Nonenablement

The references cited by the Examiner do not support the enablement rejection. They are alleged to illustrate unpredictability in the state of the art. However, the references are irrelevant and inapplicable to assessing the predictability of Applicants' invention because they pertain to the predictability of forming *liposomes* instead of the claimed *transfection particles*. Moreover, the references' teachings actually support the enablement of Applicants' invention.

A *prima facie* case of nonenablement has not been made because Applicants' claims are directed to *transfection particles* and not *liposomes*. A liposome is a specific, ordered array of particular molecules, typically lipids. Lipophile-nucleic acid complexes can take a variety of physical forms and the claimed transfection particle is not required to have a liposomal structure. Yet, these two terms, liposome and transfection particle, have been interchangeably used throughout the rejection. For example, the Examiner states that the claimed "functions are generic to all types of liposomes known in the art for administration of nucleic acids to cells in culture or in whole organisms, but the art teaches selective success of different types of liposomes for delivery of nucleic acids. . . ." *Id.* at page 7, lines 5-8. It was also alleged that the "instant claims also have important functional limitations regarding how the claimed liposomal compositions are made." *Id.* at lines 10-11. The claims pertain to transfection particles formed by complexing nucleic acid with cationic precursor molecules and subsequently dimerizing or oligomerizing the molecules. Liposomal structure is not recited in the claims. Hence, predictability in the liposomal art is not relevant or applicable to whether the claimed transfection particles are enabled.

Nonetheless, the rejection alleges that formation and use of the claimed transfection particles are unpredictable based on references pertinent only to the liposomal art. The titles of Staatz *et al.*, *Liebigs Ann. Chem.* 51-57 and 127-131 (1989) ("Staatz *et al.*"), Schott *et al.*, *Biochim. Biophys. Acta 940*: 127-135 (1988) ("Schott *et al.*"), and Zelphati *et al.*, *J. Lipos. Res.* 7:31-49 (1997) ("Zelphati *et al.*") clearly indicate that these references describe liposomes. The teachings of Freeman *et al.*, *Pharm. Res.*

13: 202-209 (1996) ("Freeman *et al.*") are also clearly directed to liposomes. *See* Freeman *et al.*, page 203, left column, second sentence of "DNA Formulations" section. Hence, these references are irrelevant and inapplicable to any determination of the predictability of Applicants' claimed transfection particles.

In summary, the Examiner has cited references in support of the § 112 enablement rejection that are irrelevant and inapplicable to Applicants' invention. The Examiner alleges to present "a review of the liposome art at the time the invention was made, especially a review of the art directed to use of SH bonds and oxidation as a mechanism for formation of the [claimed] liposome composition. . . ." PTO File Wrapper Paper No. 14, page 15, first full paragraph. However, such a review is irrelevant and inapplicable as Applicants' invention is directed to transfection particles and not liposomes. Moreover, the references do not discuss the use of SH bonds to form liposomes. Staatz *et al.* discusses thioethers incapable of dimerizing or oligomerizing; and Schott *et al.* pertains to inactive sulfurs on liposomal detergents which are incapable or prevented from forming intermolecular detergent bonds, and are ultimately used to form intermolecular bonds with antibodies. *See* Staatz *et al.*, page 51; and Schott *et al.*, Abstract and page 128, first column, first full paragraph: "We prepare liposomes which carry inactive sulfhydryl residues while the antibodies assigned for coupling are provided with the reactive sulfhydryl groups."

Accordingly, the Examiner has not set forth a *prima facie* showing that the claims are not enabled. Applicants respectfully request that the Examiner reconsider and withdraw the § 112 rejection as it applies to the claims.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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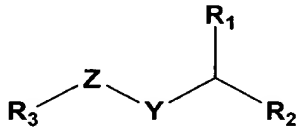
Version with markings to show changes made

Claims 34-36, 42-44 and 47 were cancelled.

1. (twice amended) A transfection particle [for transfecting higher eucaryotic cells with nucleic acid molecules *in vitro* and *in vivo*] comprising one or more nucleic acid molecules condensed by organic cationic molecules, said particle being obtained by (1) condensing said one or more nucleic acid molecules with identical or different organic cationic precursor molecules without crosslinking any of said one or more nucleic acid molecules, and (2) thereafter linking the precursor molecules to each other with one or more covalent bonds, wherein the linked precursor molecules remain [on the] condensed on said one or more nucleic acid molecules.

8. (twice amended) The transfection particle of claim 1, wherein the organic cationic precursor molecule is represented by general formula I



(I)

wherein

R_1 denotes (C_1-C_{10}-alkylene)-SH, wherein the alkylene radical may represent a straight chained or branched hydrocarbon;

R_2 denotes $-NR_4R_5$, $-NHR_4R_5^+$, $-N(R_4)_2R_5^+$, $-C(=NR_4)NR_5R_6$, guanidyl, ornithylamino, or -C(=X)-C_1-C_{10}-alkylene, wherein the alkylene radical may represent a straight chained or branched hydrocarbon and may be substituted by up to four [dialkyl amino groups] amino radicals $-NR_4R_5$ or a thiomonosaccharide;

R_3 denotes C_5-C_{30}-alkyl, straight chained or branched and optionally substituted with one or more halogen atoms or dialkyl amino groups, or
C_5-C_{30}-alkenyl, straight chained or branched having up to ten C=C-double bonds and is optionally substituted with one or more halogen atoms or dialkyl amino groups, or
C_5-C_{30}-alkynyl, straight chained or branched having up to ten C≡C-triple bonds and is optionally substituted with one or more halogen atoms or dialkyl amino groups, or
C_6-C_{10}-aryl optionally substituted, or
C_7-C_{16}-aralkyl optionally substituted, or a
C_5-C_{30}-alkyl-chain interrupted by up to 10 amino groups $-NR_4-$ and having optionally an amino-group which is optionally substituted by an amino acid;

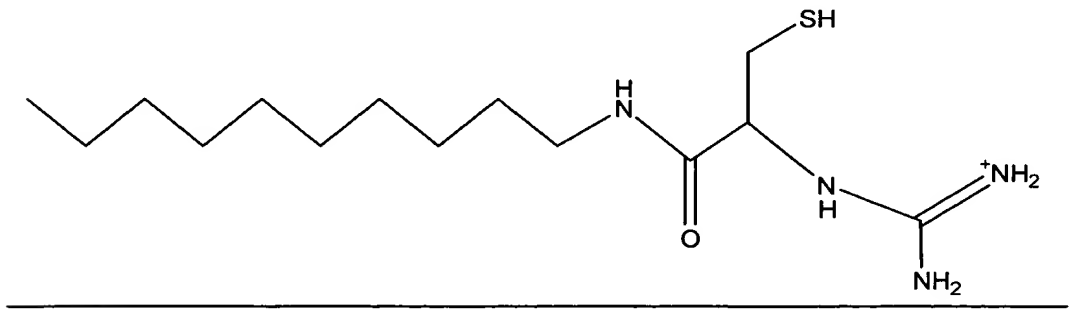
R_4, R_5 and R_6 denote independently from each other hydrogen or
C_1-C_4-alkyl;

C_5-C_{15}-alkyl chain interrupted by up to 7 amino groups -NR_4- and having optionally an amino group which is optionally substituted by the amino acid cysteine;
R_4, R_5 and R_6 denote independently from each other hydrogen or methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl or tert-butyl;
X denotes O or S;
Y denotes C=O or C=S and
Z denotes O, S or -NR_4-.

11. (twice amended) The transfection particle of claim 8, wherein the cationic precursor molecules correspond to the general formula I, wherein
R_1 denotes -CH_2-SH;
R_2 denotes -NH_2, -NH_3^+, -$C(=N^+H_2)NH_2$, guanidyl, ornithylamino, or -C(=O)-C_1-C_4-alkyl straight chained or branched and optionally substituted with F, Cl, Br or -NH_2,
or an ornithine radical or a S-galactosyl radical;
R_3 denotes a C_6-C_{15}-alkyl radical straight chained or branched and optionally substituted with F, Cl, Br or -NH_2;
Y denotes C=O;
Z denotes O or -NH-.

12. (twice amended) The transfection particle of one of claims 8 to 11, wherein R_2 is guanidine [ornithine] or ornithylamino.

15. (once amended) The transfection particle of claim 14, wherein the cationic molecule [is N-decyl-2-guanidinium-cysteine] has the following formula:



16. (once amended) The transfection particle of one of claim 8 to 11, wherein R_1 is a methylenethiol, R_2 is an [ornithine] ornithylamino, R_3 is a decane, Y is a carbonyl, Z is an amine, and pharmaceutically acceptable salts thereof.

X denotes O or S;
Y denotes C=O or C=S and
Z denotes O, S or $-NR_4-$.

9. (twice amended) The transfection particle of claim 8, wherein the cationic precursor molecules correspond to general formula I, wherein

R_1 denotes (C_1-C_6-alkylene)-SH, wherein the alkylene radical may represent a straight chained or branched hydrocarbon;

R_2 denotes $-NR_4R_5$, $-NHR_4R_5^+$, $-N(R_4)_2R_5^+$, $-C(=NR_4)NR_5R_6$, guanidyl, ornithylamino, or $-C(=X)-C_1-C_4$-alkylene, wherein the alkylene radical may represent a straight chained or branched hydrocarbon and may be substituted by up to four amino radicals $-NR_4R_5$ or a thiomonosaccharide;

R_3 denotes C_5-C_{20}-alkyl, straight chained or branched and optionally substituted with F, Cl, Br or $-NR_4R_5$, or
C_5-C_{20}-alkenyl, straight chained or branched having up to five C=C-double bonds and is optionally substituted with F, Cl, Br or $-NR_4R_5$, or
C_5-C_{20}-alkynyl, straight chained or branched having up to five C≡C-triple bonds and is optionally substituted with F, Cl, Br or $-NR_4R_5$, or
C_6-C_{10}-aryl optionally substituted with C_1-C_4-alkyl, F, Cl, Br or $-NR_4R_5$, or
C_7-C_{14}-aralkyl optionally substituted with C_1-C_4-alkyl, F, Cl, Br or $-NR_4R_5$, or
a C_5-C_{20}-alkyl chain interrupted by up to 10 amino groups $-NR_4-$ and having optionally an amino group which is optionally substituted by an amino acid;

R_4, R_5 and R_6 denote independently from each other hydrogen or C_1-C_4-alkyl;

X denotes O or S;
Y denotes C=O or C=S and
Z denotes O, S or $-NR_4-$.

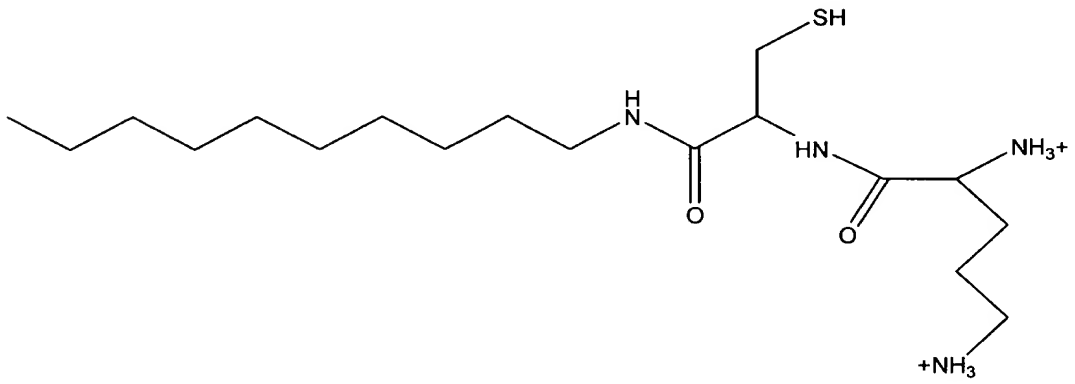
10. (twice amended) The transfection particle of claim 8, wherein the cationic precursor molecules correspond to general formula I, wherein

R_1 denotes (C_1-C_4-alkylene)-SH, wherein the alkylene radical may represent a straight chained or branched hydrocarbon;

R_2 denotes $-NR_4R_5$, $-NHR_4R_5^+$, $-N(R_4)_2R_5^+$, $-C(=NR_4)NR_5R_6$, guanidyl, ornithylamino, or $-C(=X)-C_1-C_4$-alkyl, wherein the alkyl radical may represent a straight chained or branched hydrocarbon and may be substituted by up to four amino radicals $-NR_4R_5$, or a thiomonosaccharide;

R_3 C_5-C_{12}-alkyl, straight chained or branched and optionally substituted with F, Cl, Br or $-NH_2$, or a

17. (once amended) The transfection particle of claim 16, wherein the cationic molecule [is N-decyl-2-ornithinyl-cysteine] has the following structure:



18. (once amended) The transfection particle of one of claim 8 to 10 [11], wherein the monosaccharide which is bonded via a sulfur atom is selected from the group consisting of galactose, lactose, glucose, arabinose , fructose, sorbose, xylose, ribose, mannose each of them in their D- or L-form.

22. (thrice amended) The transfection particle of claim 1, wherein the one or more covalent bonds between the cationic molecules are degradable under [cellular] reductive or slightly acidic conditions, or in the presence of enzymes.

27. (thrice amended) The transfection particle of claim 1, characterized in that it is linked via one or more covalent bonds to one or more members of the group consisting of protein ligands, sugar residues, fusogenic peptides, viruses, adenoviruses, and combinations thereof [one or more cellular targeting functionalities and/or one or more functionalities capable of facilitating endocytosis].

28. (twice amended) The transfection particle of claim 27, wherein said [functionalities] one or more members of the group are linked via said one or more covalent bonds to the cationic molecules.

29. (twice amended) The transfection particle of claim 27, wherein said one or more members of the group [functionalities] are linked via said one or more covalent bonds to nucleic acid binding molecules that are present in addition to the cationic molecules.

30. (twice amended) The transfection particle of claim 27, wherein [the targeting functionality is a cellular] said one or more members of the group is a protein ligand.

31. (twice amended) The transfection particle of claim 27, wherein [the targeting functionality is a] said one or more members of the group is a sugar residue.

32. (once amended) The transfection particle of claim 31, wherein the sugar residue is galactose.

33. (once amended) The transfection particle of claim 31, wherein the sugar residue is mannose.

37. (once amended) The transfection particle of claim 27 [34], wherein said one or more members of the group [the endosomolytic function] is a fusogenic peptide.

38. (once amended) The transfection particle of claim 27 [34], wherein said one or more members of the group [the endosomolytic function] is a virus.

45. (twice amended) A kit of parts comprising one or more nucleic acid molecules, one or more cationic precursor molecules, suitable buffers, and other reagents or mechanical devices that are useful for preparation[,] or purification [and *in vitro* or *in vivo* application] of a transfection particle of claim 1.

46. (twice amended) The kit of parts of claim 45 comprising in addition [or more functionality for cellular targeting] one or more members of the group consisting of nucleic acid binding molecules that are present in addition to the cationic molecules, protein ligands, sugar residues, fusogenic peptides, viruses, adenoviruses, and combinations thereof.

48. (once amended) A transfection particle [for transfecting higher eucaryotic cells with nucleic acid molecules *in vitro* and *in vivo*] comprising:

a) one or more nucleic acid molecules;

b) identical or different organic cationic precursor molecules linked to each other via one or more covalent bonds;

wherein said precursor molecules are ionically associated with said one or more nucleic acid molecules without forming any crosslinks between said nucleic acid molecules and said cationic precursor molecules, thereby condensing said one or more nucleic acid molecules.